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Biochemistry

OCCURRENCE OF BROAD SPECTRUM ANTIBACTERIAL AGENTS IN ROOTS OF PAEONIA OFFICINALIS L.

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ABSTRACT Folkloric medicinal application of Paeonia officinalis L. (Family: Paeoniaceae) in the inhibition of bacteria was investigated. Phytochemical analysis revealed the presence of carbohydrates, glycosides, steroids, triterpenes, alkaloids,tannins and saponins. Hexane (HE), dichloromethane (DCM), ethyl acetate (EA) and methanol (ME) extracts of P. officinalis L. roots were evaluated for antibacterial activity, against pathogenic bacteria, Shigella dysenteriae, Salmonella typhi, Corynebacterium ulcerans, Klebsiella pneumonia, Staphylococcus aureus, Methicillin resistant Staphillococus aureus (MRSA), Streptococcus pyogens, Baccillus cereus, Escherichia coli and Enterobacter sp, using the agar-in-well diffusion method. Determination of zone of inhibition (ZI) showed inhibition ranging from 20-23 mm (HE), 25-30 mm (DCM), 30-33 mm (EA) and 22-25 mm (ME) against the entire test organisms except Methicillin resistant Staphillococus aureus (MRSA), Klebsiella pneumonia and Shigella dysenteriae. The results of the minimum inhibitory concentration (MIC) showed that EA fraction inhibited the growth of all test organisms at a low concentration of 5 mg/ml. Higher MIC values were observed for DCM (5-10 mg/ml), HE and ME fraction all showed MIC at 10 mg/ml. The microorganisms were completely killed at a higher concentration; EA (MBC/MFC; 10 mg/ml), DCM (MBC/MFC; 10-20 mg/ml), ME and HE (MBC/MFC; 20 mg/ml). Bacterial evaluation reveals that the HE extract had the highest activity with MIC of 0.675 mg/ml, followed by DCM extract. The results clearly showed that the plant had potential that can be explored in the search for anti-bacterial drug.

KEYWORDS : P. officinalis L., E. coli, B. cereus, S pyogens, S. aureus, Antibactrial evaluations, and Phytochemical screening.

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Chandra, 2013), for centuries, many plant compounds have an outstanding role in medicine. Their pharmac ological and economical values are of great importance. They are either used directly or after certain chemical modification processes. These medicinal plants contain bio active compounds (Sasidharan et al., 2010), have been exploited in ayurvedic medicines for the treatment of various ailments. The prevalence of bioactive principles such as tannins, terpenoids, flavonoids, alkaloids, proteins, saponins and steroids etc. underscores the needs for continuous search for active ingredients extracted from plant, which can be used as antibiotic drugs (Ojiako, 2014).

Bacterial infections are currently a major health hazard due to multidrug-resistant forms of bacilli (Ramachandran et al., 2014). Global efforts are underway to eradicate such infections using new drugs with new modes of action, higher activity, and fewer side effects in combination with vaccines. For this reason, unexplored new sources need to be examined, to develop natural drugs from these new sources (Murtaza et al., 2016). Since ancient times, different plant part extracts have been used as traditional medicines against diseases including infections. This knowledge may be useful in developing future powerful drugs. Plant derived natural products are again becoming important in this regard. In an effort to expand the spectrum of antibacterial agents from natural resources, *P. officinalis L.* belongs to family Paeoniaceae.

In the current investigations carried out, a screening of the methanol, ethyl acetate, dichloromethane and hexane root extracts of *P. officinalis L.* against pathogenic bacteria, in order to detect new sources of antibacterial agents.

MATERIALS AND METHODS

Plant materials

The plant material of P. officinalis L. root the plant root was air-dried under shade, crushed to make powder and was stored away from moisture until needed.

Extraction of plant materials

The pulverized leaves *P. officinalis* (500 g) was carefully weighed and macerated with 95% methanol for one week. The extract was decanted and filtered. The process was repeated three times for exhaustive extraction. The three sets of extracts were combined on confirmation by TLC. The combined methanol extract was partitioned with hexane, dichloromethane and ethylacetate. The extracts were concentrated in vacuum at 40˚C using rotator evaporator and later subjected to air drying to give dried crude extracts.

Phytochemical screening

The hexane, dichloromethane, ethyl acetate and the methanol extracts of the plant was subjected to phytochemical screening using standard techniques (Harborne, 1973). The metabolites tested for included, carbohydrates, tannins, saponnis, flavonoids, anthraquinones, glycosides, steroids, terpenes and alkaloids.

Antimicrobial studies

The antimicrobial activities of the HE, DCM, EA and ME extracts and standard drugs (Ciprofloxacin and Sparfloxacin) were determined using microbial strains. The test microorganisms used are *Shigella dysenteriae, Salmonella typhi, Corynebacterium ulcerans, Klebsiella pneumonia, Staphylococcus aureus, Methicillin resistant Staphil locococus aureus, Streptococcus pyogens, Baccillus cereus, Escherichia coli and Enterobacter sp.* The well diffusion method of Preeti et al., (2014), was used to determine the antibacterial activity of the test extracts. Pure cultures of the bacterial organisms were inoculated on to Mueller Hinton Agar (MERCK) and incubated for 24 h at 38oC. About 5 discrete colonies were aseptically transferred using sterile wire loops into tubes containing sterile normal saline (0.85% NaCl) and were adjusted to a turbidity of 0.5 Mac Farland Standard. The suspensions were then inoculated on the surface of sterile Mueller – Hinton Agar plates using sterile cotton swabs. A sterile 6 mm diameter Cork borer was used to make holes (wells) into the set of inoculated Mueller-Hinton Agar. The wells were filled with different concentration of the test extracts. The plates were incubated for 24h at 38oC. All the tests were performed in triplicate and the antibacterial activities were determined as mean diameters of inhibition zone (mm) produced by the test compounds.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) were determined for the extracts using micro broth dilution method in accordance with (Vollekova etal., 2001). Serial dilution of the least concentration of the extracts that showed activity were prepared using test tubes containing 9 ml of double strength nutrient broth (OXOID). The test tubes were inoculated with the suspension of the standardized inocula and incubated at 38 oC for 18 h. Minimum inhibition

Concentrations (MIC) were recorded as the lowest concentrations of the compounds showing no visible growth (turbidity) in the broth.

Minimum Bactericidal Concentration (MBC/MFC)

The minimum bactericidal concentration was determined by

aseptically inoculating aliquots of culture, from the minimum inhibition concentration (MIC) tubes that showed no growth, on sterile nutrient Agar (OXOID) plates and incubated at 38oC for bacteria for 48 h. The MBC/MFCs were recorded as the lowest concentration of extracts showing no bacterial growth at all.

RESULTS AND DISCUSSIONS

Phytochemical analysis

P. officinalis belongs to family Paeoneaceae it is an herbaceous paraneal plant have compound, deeply lobed leave with large fragrant flower it is a garden plant and well known for treatment of inflammation, painful conditions, haemorrhoids, fissures, fistula and ulcer problems and also used as lever tonic.

Phytochemical screening (Table 1) of the crude methanol, ethyl acetate, dichloromethane and hexane extracts revealed the presence of carbohydrates, glycosides, alkaloids, tannin, flavonoids, Saponins, steroids and triterpenes. These phytochemicals could be responsible for the antimicrobial activities exhibited by the extract and hence justify the ethnomedicinal uses of *P. officinalis L.*

Antimicrobial screening

The antimicrobial activity of the extract showed that all the extracts exhibited moderate to good antibacterial activity against all the pathogens tested except *Methicillin* resistant *Staphillococus aureu*s (MRSA), *Klebsiella pneumonia, Shigella dysenteriae* (Table2). The ethyl acetate extract exhibited the highest zone of inhibition (33 mm) against Bacillus cereus and Salmonella typhi. Whereas hexane extract exhibited the lowest zone of inhibition (20 mm) against *Corynebacterium ulcerans* and *Salmonella typhi* (Table3). The ethyl acetate extracts exhibited minimum inhibitory concentration (MIC) 5mg/ml against all microorganisms (Table 4.) The MBC showed that the ethyl acetate extract was bactericidal at 10mg/ml against all test microorganisms (Table 5.)

Metabolites	HE	DCM	EA	ME
Triterpenes	+	+	-	+
Glycosides	+	+	+	+
Alkaloids	-	+	+	+
Flavonoids	-	+	+	+
Anthraquinones	-	-	-	-
Steroids	+	+	-	+
Carbohydrate	-	+	+	+
Tannins	-	+	+	+
Saponins	-	-	-	+

Table 1. Phytochemical Analysis.

Key: + = present, - = absent, HE = hexane extract, DCM = dichloromethane extracts, EA = Ethyl acetate extracts, ME =Methanol extracts

Table 2: Sensitivity test of extracts and standard drugs.

Test Organisms	DCM	EA	ME	HEX	CFX	FCZ
Salmonella typhi	S	S	S	S	S	R
Shigella dysenteriae	R	R	R	R	S	R
Klepsiella pneumoniae	R	R	R	R	S	R
Enterobactor sp	S	S	S	S	R	R
Escherichia coli	S	S	S	S	S	R
Methicillin Resistant S. aureus	R	R	R	R	S	R
Staphylococcus aureus	S	S	S	S	S	R
Streptococcus pyogenes	S	S	S	S	S	R
Bacillus cereus	S	S	S	S	S	R
Corynebacterium ulcerans	S	S	S	S	R	R

Key: S= Sensitive, R = Resistance

Table 3. Zones of Inhibition (mm) of the extracts and standard drugs

Test organisms	DCM	EA	ME	HEX	CFX	FCZ
Corynebacterium ulcerans	25	29	22	20	0	0
Salmonella typhi	29	33	24	20	41	0
Shigella dysenteriae	0	0	0	0	39	0
Klepsiella pneumoniae	0	0	0	0	40	0
Enterobactor sp	26	30	25	21	0	0
Escherichia coli	27	30	24	22	32	0
Methicillin Resistant S. aureus	0	0	0	0	35	0
Staphylococcus aureus	28	32	24	22	37	0
Streptococcus pyogenes	27	30	24	23	35	0
Bacillus cereus	30	33	25	21	40	0

Key:

Table 4. Result of Minimum Inhibitory Concentration (MIC)

Test Organisms	DCM	EA	ME	HE
Salmonella typhi	5.0	5.0	10.0	10.0
Enterobacter sp	10.0	5.0	10.0	10.0
Escherichia coli	5.0	5.0	10.0	10.0
Staphylococcus aureus	5.0	5.0	10.0	10.0
Streptococcus pygenes	5.0	5.0	10.0	10.0
Bacillus cereus	5.0	5.0	10.0	10.0
Corynebacterim ulcerans	10.0	5.0	10.0	10.0

Key: DCM – Dichloromethane, EA – Ethyl acetate, ME – Methanol, HE – Hexane

Table 5. Minimum bactericidal concentration (MBC) of the extra cts in (mg/ml).

Test Organisms	DCM	EA	ME	HE
Corynebacterimulcerans	20.0	10.0	20.0	20.0
Salmonella typhi	10.0	10.0	20.0	20.0
Enterobactersp	20.0	10.0	20.0	20.0
Escherichia coli	10.0	10.0	20.0	20.0
Staphylococcus aureus	10	10	20.0	20.0
Streptococcus pygenes	10.0	10.0	20.0	20.0
Bacillus cereus	10.0	10.0	20.0	20.0

Key: DCM = Dichloromethane, EA = Ethylacetate, ME =Methanol, HE =Hexane

Recently there has been considerable interest in the use of plant based drug as an alternative method to control pathogenic microorganism (Aqil et. al., 2005) and many components of plants products have been shown to be specially targeted against resistant pathogenic bacteria (Nostro et. al., 2006). The emergence of multidrug resistant strain of many pathogens is a serious threat and makes chemotherapy more difficult. Moreover, the current cost of most of the chemotherapeutic agents is unbearable to the public especially in developing countries (Chandra, 2013). Therefore attempts must be made towards the development of effective natural, non-toxic drug for treatment. The present work was done to explore the antimicrobial property of *P. officinalis L.,* a medicinal plant used for treatment of nausea, fever, cough generalized body pains ulceration, hemorrhoids, fissures, fistula, abscess and itching.

Phytochemical analysis carried out on the plant extracts revealed the presence of constituents which are known to demonstrate medicinal as well as physiological activities (Jain and Bari 2010).Phytochemical screening of the plant extracts revealed the presence of phytochemicals such as carbohydrates, tannins, saponins, glycosides, steroid, triterpenes and alkaloids. These could be responsible for high antimicrobial activity by the plant extracts. Tannins, saponins and alkaloids have been reported to have pronounced physiological effect particularly on the nervous system (Simkin et al 2008).Tannins encompass a heterogeneous group of compounds and polymers (polyphenols). In general their non-specific activity has been ascribing to their ability to complex metal ions, scavenge radicals and reduce active oxygen species and form tight complexes with a wide array of proteins and polysaccharides (Haslam 1996). Hence, they have antioxidative properties. Saponins are known to produce inhibitory effect on inflammation (Just et al 1998) Steroid and Triterpenes have been reported to have antibacterial properties (Raquel 2007).

Alkaloids have been reported for their cytotoxic, analgesic, antisplasmodic and antibacterial (Okwu 2004) properties. Glycosides are known to lower the blood pressure according to many reports (Nyarko and Addy). The presence of these phytochemicals in *P. officinalis L.* extracts validates the claim by the traditional healers in the treatment of several ailments. The antimicrobial sensitivity test of the root extracts of *P. officinalis L.* showed that the extracts have moderate to good activity. The determination of zone of inhibition (ZI)

showed inhibition ranging from 20-23 mm (HE), 25-30 mm (DCM), 30-33 mm (EA) and 22-25 mm (ME) against the entire test organisms except *Methicillin resistant Staphillococus aureus (MRSA)*, *Klebsiella pneumonia* and *Shigella dysenteriae.* The ethyl acetate extract had the highest zone of inhibition of 33mm against Bacillus cereus and salmonella typhi. The results of the minimum inhibitory concentration (MIC) showed that EA fraction inhibited the growth of all test organisms at a low concentration of 5 mg/ml. Higher MIC values were observed for DCM (5-10 mg/ml), HE and ME fraction all showed MIC at 10 mg/ml. The microorganisms were completely killed at a higher concentration; EA (MBC/MFC; 10 mg/ml), DCM (MBC; 10-20 mg/ml), ME and HE (MBC; 20 mg/ml). Antituberculosis evaluation reveals that the hexane extract had the highest activity with MIC of 0.675 mg/ml.

CONCLUSION

The extracts of *P. officinalis L.* were found to inhibit bacterial strains. The present study justified the claimed uses of root of this plant in the traditional system of medicine to treat various infectious disease caused by tested microbes. To the best of our knowledge this is the first research done on this plant *P. officinalis L*. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as antimicrobial agents. The present results will form the basis for selection of the plant species for further investigation for the potential discovery of new natural bioactive compounds.

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